INFECTION EXPERIMENTS WITH WART DISEASE OF POTATOES. *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.), PERC.

BY

MARY D. GLYNNE, M.Sc.

REPRINTED FROM
THE ANNALS OF APPLIED BIOLOGY
VOL. XII, NO. 1, FEBRUARY 1925

CAMBRIDGE
AT THE UNIVERSITY PRESS

PRINTED IN GREAT BRITAIN

[From THE ANNALS OF APPLIED BIOLOGY, Vol. XII, No. 1, February, 1925.]

INFECTION EXPERIMENTS WITH WART DISEASE OF POTATOES. *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC.

By MARY D. GLYNNE, M.Sc.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With 1 Text-figure.)

CONTENTS.

INTRODUCTION.

Investigations directed towards the control of wart disease have hitherto been carried out along two main lines. On the one hand the ultimate aim has been to destroy the parasitic organism *Synchytrium endobioticum* in the soil and so eliminate the disease, and, on the other hand, to render it innocuous by cultivating varieties of potato immune to the disease. It has been clearly shown that the winter sporangia of the parasite survive in the soil for many years in the absence of the potato plant, so that the disease cannot be eradicated from the soil by

any system of crop rotation. Soil sterilisation is the one method of destroying the parasite that seems to offer any hope of success. Although such treatment has often been attempted and has given mostly negative results, in a few cases successful soil sterilisation has been achieved. This has not yet proved feasible on a large scale, but its possibilities are now being extensively studied. At present the only practicable method of controlling wart disease is to grow immune varieties of potato. Plant breeders are therefore actively engaged in trying to produce new types which shall combine the good qualities of some of the best susceptibles, such as early maturity, high yield, good flavour and cooking quality, with immunity. Every year some hundreds of new varieties raised from seed are tested for immunity to wart disease at potato testing stations, such as that at Ormskirk, in Lancashire.

The varieties which prove susceptible are discarded, while the immunes are further tested and the best of them selected to be put on the market.

The study of wart disease of potatoes, whether it is directed towards soil sterilisation or towards the production of new immune varieties, and the latter includes the fundamental study of the genetics of immunity to wart disease, has hitherto been very seriously hampered by the lack of effective experimental methods. In work on soil sterilisation the rational sequence of study whereby conditions, chemical and physical, which bring about the death of the sporangium are determined first *in vitro*, then on a pot scale, and lastly in the field, has not been possible. The difficulty experienced in inducing germination of even a small percentage of sporangia under observation and so of testing their viability rendered *in vitro* tests impracticable.

Pot experiments, though they have been used to a limited extent in soil sterilisation work have proved so unreliable that they have been largely discontinued. In some instances good results involving infection by wart disease of every control plant, have been obtained in pots during spring and summer, the normal season for infection, but in other cases at the same time of the year under apparently similar or equally favourable conditions, little or no infection has been found. For instance, potatoes in pot experiments put up by Gimingham and Spinks (3) in the summer of 1919 were all well infected. At Rothamsted in two successive summers, pot experiments carried on out-of-doors showed in the first season infection in every control plant and in the second, complete absence of infection. In 1920 a set of experiments in a glasshouse at the Potato Testing Station at Ormskirk failed completely because very little

infection appeared even in the controls. Again, in 1921, two sets of experiments involving altogether about 200 pots, each holding 60 lb. of soil, put up by Dr W. B. Brierley, Mr W. A. Roach and myself in a glasshouse, had respectively only three out of seven and six out of ten control plants infected and these for the most part not heavily. Similar experiments carried out in the open in 1922 failed completely through lack of infection and yet the soil used in all these cases came from a plot of land at Ormskirk regarded as one of the most heavily infected areas in the world. Hitherto, therefore, pot experiments have been of little use in research on soil sterilisation and thus a relatively simple and economical method of selecting the best chemicals for testing out on a field scale has not been available.

The only method which is regarded as reliable for testing the immunity or susceptibility of new varieties of potato involves growing them in the field. At Ormskirk the standard method is to grow forty tubers of each variety in field plots on land heavily infected with wart disease. Those which show no infection the first year are cultivated again in the following season and if again free from wart disease are officially listed as immune. If as in the hot dry summer of 1921 and to a lesser extent in 1923, seasonal conditions are unfavourable to the development of the disease infection may be so slight that the tests must be repeated in full or in part the following year. In a year such as 1924, however, when the climatic conditions were exceptionally favourable for the development of wart disease the average infection of Arran Chief, one of the most susceptible varieties of potato in certain heavily infected trial plots at Ormskirk, was about 80 per cent.

The method of field testing for immunity has up to the present proved a reliable one, but its great disadvantage lies in the length of time and expense involved. At least one year is required to raise the necessary tubers from seed after which susceptibility may be shown the following season, but immunity cannot be proved in less than two and in the case of an unfavourable season, three years. A more rapid method of testing the immunity or susceptibility of varieties and tubers is therefore needed.

In the absence of any anatomical criterion or known biochemical reaction which might serve as an index to immunity, the direct method of actual infection with *Synchytrium* is the only means for testing this quality. The standardisation of some method for effecting this more rapidly than is done in the field and by a method less subject to seasonal variation is thus required.

The present communication embodies the results of a somewhat empirical study of some of the conditions and factors controlling infection by wart disease of potatoes. The work is related to and to a large extent has directly grown out of the practical needs and difficulties in a larger study of soil sterilisation with regard to wart disease in progress at Rothamsted. The possibility has been kept in view that by discovering the conditions that favour infection a reliable method for infecting susceptible tubers might be obtained, in the first place, to provide a method of pot experimentation as a laboratory basis for soil sterilisation work and, secondly, to provide a quick method of testing immunity or susceptibility to wart disease.

The communication is divided into two parts, the first of which treats of infection of the host brought about by winter sporangia in the soil, and the second of infection by means of summer sporangia in the laboratory. The work is incomplete but the possibility that the results might be of immediate use in other directions, particularly in the testing of immunity or susceptibility, appeared to justify publication at the present stage.

INFECTION BY WINTER SPORANGIA IN THE SOIL.

TECHNIQUE.

Throughout these experiments, except where otherwise definitely stated, the following procedure was adopted:

Tubers of Arran Chief, one of the most susceptible varieties of potato known, were grown singly in ordinary flower pots with a top diameter of 7 inches, each holding about 3 lb. of soil. These pots were found to be large enough to allow of moderate growth, while they were small enough to be conveniently used in large numbers[1]. Each pot stood in a saucer of 7 inches diameter. The soil used consisted of a mixture of heavy field soil from Rothamsted, with sand, and an organic material consisting of broken chaff and straw and known as "cavings," all passed through a sieve with a $\frac{1}{4}$-inch mesh and mixed in the following proportions:

Soil	...	...	37·5 per cent.
Sand	...	...	50 ,,
Cavings	...	...	12·5 ,,

[1] At Ormskirk in 1921 and 1922 large glazed pots 12 × 12 inches each holding 60 lb. of soil were used by Dr W. B. Brierley, Mr W. A. Roach and myself. Growth though possibly better than in the 7-inch pots was not sufficiently so to make the use of the former worth while in view of the far greater cheapness and convenience of the small pots and their satisfactory experimental results.

The sporangial material which was collected in the late summer and early autumn consisted of warts together with a certain amount of attached decomposed tuber. This material was air-dried, broken up in a mincing machine, passed through a sieve of 3 mm. mesh and mixed well together. A definite quantity, measured by volume, of sporangial material was added to each of 15 lb. or 30 lb. of the soil mixture described above, and well incorporated.

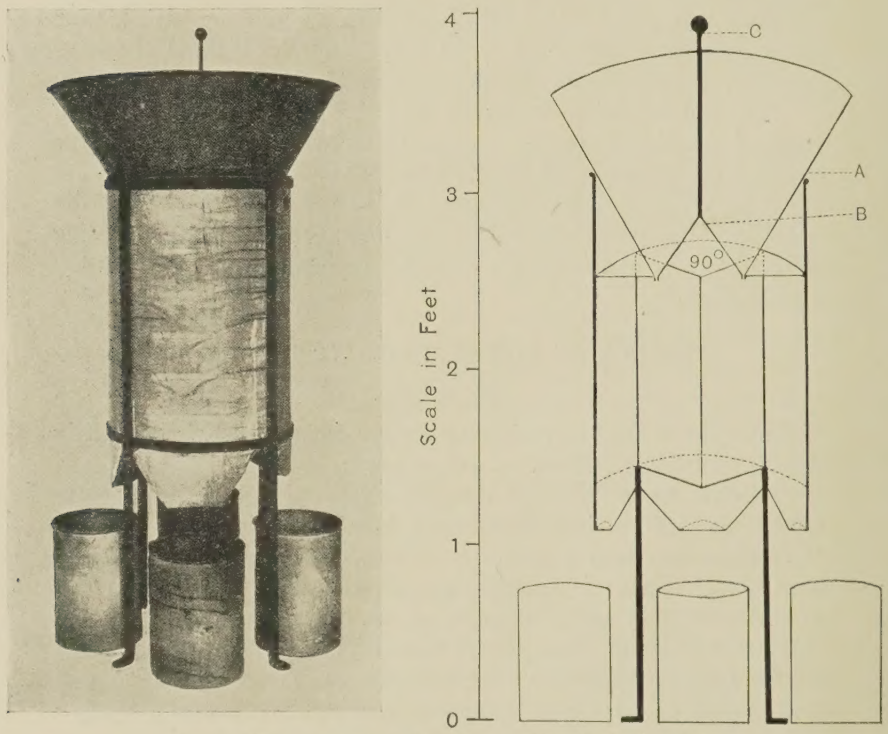

Fig. 1. Mixing apparatus with diagram of longitudinal section.

In the earlier experiments the sporangia were mixed with the soil by hand, but later a less laborious and more efficient method was adopted by use of the soil sampler shown in Text-fig. 1[1]. It is constructed of galvanised iron and consists of a main cylinder divided by means of two

[1] I am indebted to Dr B. A. Keen for drawing my attention to this type of apparatus, which has previously been used in certain sampling operations, and for suggestions as to the manner of construction.

diametrical plates into four quarters each of which leads into a basal spout. A funnel *A*, placed on top of the main cylinder, has its lower end blocked by means of a conical plug *B* attached to a rod *C*. The soil and sporangia are put into *A* which will hold about 30 lb. of soil. *B* is then drawn upwards by means of *C* and the soil and sporangia fall and are divided by the plates into four portions which issue from the spouts into receptacles. The four portions of the soil are then returned to the funnel *A*, after replacing *B*, and the process repeated. Thorough and homogeneous mixing was found to have taken place when this had been performed six times. In this way the soil in large numbers of pots could be infected rapidly to about the same extent.

Three pounds of infected soil and one tuber were put into each pot. Watering was always effected by putting the water into the saucers and not directly on to the soil. It was found that when the soil was kept very wet during the first few weeks the sprouting tubers showed a tendency to decay. For the first two to four weeks, therefore, until the shoots appeared above the surface, the soil was kept fairly dry. For the remaining period, except when otherwise definitely stated, the soil was kept very wet by keeping water practically always standing in the saucers.

The time allowed between setting the tubers in infected soil and examining the plants for wart disease was generally about twelve weeks. This was found sufficient to permit of infection followed by considerable growth of warted tissue.

Relation of External Physical Conditions to Infection.

General field observation indicates that temperature and moisture are likely to play an important part in controlling infection of the potato plant by the wart disease organism. Temperature investigations carried out in America (6), using controlled soil temperature tanks, show that infection does not occur above 22° C., indicating a relatively low temperature requirement.

(a) *Moisture.*

The effect of varying moisture conditions on infection was studied empirically in the following way: soil, equally and homogeneously infected, was put into 40 pots and one Arran Chief tuber planted in each. The soil in 10 pots (Pots 21–30) was kept moist, rather wetter than was considered optimum for the potato plant. Daily inspection showed when these required watering, which was taken to be when the water standing in the saucers had been absorbed by the soil. Water was then applied

through the saucers to the soil in these 10 and the 30 other pots in the following quantities:

Pots 1–10 received 25 c.c. per pot.
11–20 ,, 50 ,, ,,
21–30 ,, 100 ,, ,,
31–40 ,, 200 ,, ,,

The soil was thus kept "dry," "fairly dry," "moist" and "very wet" in the different sets of pots.

Table I shows the number of plants infected in each set of ten. The absence of infection in the "dry" and "fairly dry" soils was not unexpected, but its complete absence in the "moist" soil was a little surprising. Heavy infection was however found in the "very wet" soil.

Table I.

Effect of Varying Soil Moisture on Infection[1].

Date of experiment: 25. vi. 22 to 2. x. 22. Variety of tuber: King Edward.

Pot numbers	Quantity of water per pot, per time watered c.c.	Condition of soil	Number of plants	
			Clean	Infected
1–10	25	"dry"	10	0
11–20	50	"fairly dry"	10	0
21–30	100	"moist"	9*	0
31–40	200	"very wet"	1*	8

* One out of the ten tubers planted failed to grow.

The absence of wart disease on plants grown in soil of a low or even moderate moisture content and its presence only under very wet conditions suggested a reason for many earlier failures to obtain a high percentage infection.

In 1922 potatoes grown in pots containing thoroughly infected soil in the glasshouse showed a low percentage infection (only 20 per cent.), while others set up in exactly the same way in the open had 85 per cent. of the plants well warted. In the former the soil was kept moist by moderate watering, while in the latter it was extremely wet because of the very heavy rainfall of that season. It thus appeared probable that insufficient soil moisture accounted for the low degree of infection obtained in the earlier glasshouse experiments. In the following year, therefore, pot experiments in the glasshouse were carried on in the same

[1] It may be interesting to note that infection by Rhizoctonia also increased with moisture. Practically none was found in the relatively dry soils of pots 1–20, while much Rhizoctonia was found on the plants grown in "moist" and "wet" soil (pots 21–40).

way as before except that the soil was kept very wet by having water continually standing in the saucers (after the first two or three weeks). In the open also, by means of abundant watering which was necessary in the dry season of 1923, the soil was maintained at a high degree of moisture. Heavy infection was found in 80–100 per cent. of the potato plants both in the glasshouse and out-of-doors. This was again the case in 1924. It would seem, therefore, that in previous experiments absence of sufficient soil moisture had been the limiting factor preventing infection.

The persistent appearance of an occasional clean plant under conditions which favour heavy infection in all its neighbours requires investigation. It is not impossible that the seed used, although carefully selected, may contain occasional immune individuals.

Again, 10 tubers were grown in one large pot (diameter 18 inches, height 12 inches) containing heavily infected soil kept moist in the glasshouse from December 20th, 1921, to May 1st, 1922. Growth was good but no sign of wart disease was found on any plant. The pot was moved out-of-doors and 10 new tubers planted in the same soil and grown from May 4th to September 13th, 1922, when every plant was found to be heavily infected. In the later case the soil was kept very wet by the heavy rainfall of the summer of 1922. At the same time it was thought that this result might be due to some possible "seasonal" effect which was further investigated, but, in the light of subsequent work described later in this paper, there can be little doubt that moisture was again the factor controlling infection. In subsequent experiments the soil was kept very wet and when other conditions favourable to the development of wart disease were maintained a high percentage, 80–100 per cent., of the plants were always infected. It thus appears probable that insufficient soil moisture is a common source of failure in pot experiments with wart disease.

The importance of moisture in controlling infection of potato plants by the wart disease organism, shown in the foregoing pot experiments, agrees with the common field observation that the incidence of the disease is much greater in wet seasons than in dry and that it is greater in the wetter regions, such as the northern and western counties of England than in the drier southern and eastern counties.

The very wet condition of the soil which was ascertained to be the factor determining infection by wart disease in potatoes grown in pots differs from that in the field in that it is continuous. Such a wet state of the soil exists under field conditions only at intervals during or soon

after rain. It therefore seemed possible that heavy infection, which is often found in the field, might also occur in pots when the soil was kept very wet for a part only instead of for practically the whole of the growth period. The following experiment was arranged to give some information on this question.

Seventy tubers were planted in separate pots containing freshly and uniformly infected soil. For the first four weeks all were treated alike and given, through the saucers, the same measured quantity of water which was only enough to maintain the soil in a "fairly dry" condition. At the end of this time shoots from all the tubers showed above the soil which could then be kept very wet without danger of the growth of the plants being prevented by rotting. From the fifth to the twelfth week watering was varied for each set of five pots. One set was kept "fairly dry" and two others "very wet" during the whole eight weeks, while the remaining sets severally were "very wet" for a month, a fortnight, a week, or two separate weeks, at different times. During the wet periods water was kept standing continuously in the saucers. These were emptied at the end of the wet week and no water was added for a fortnight, after which time the soil was "fairly dry." A small amount (100 c.c. per pot and once, in hot weather, 200 c.c.) was then given weekly. Except when the soil was "very wet" a "fairly dry" condition was maintained which aimed at being just wet enough to prevent wilting. The number of plants heavily infected, slightly infected, *i.e.* showing only minute traces of infection, and clean, are shown in Table II.

The five plants which were kept "fairly dry" during the whole period grew less well than the others but produced numerous small tubers quite sufficient to show infection if this had been present. The remaining plants were fairly well grown, having about four or five tubers per plant. No marked differences in the amount of subterranean growth were noted but the height of the aërial shoots varied considerably between the different sets of pots.

The variation in infection is shown in Table II. The plants grown in the "fairly dry" soil (pots 1–5) were all free from wart disease, while 10 plants grown in soil which was wet for the last eight weeks (pots 6–10 and 66–70) were all infected, most of them heavily. When the soil was wet for only one month, beginning at the fifth week (pots 11–15), all five plants were infected to almost the same extent as those which were wet for eight weeks. When, however, the wet period of four weeks began in the seventh and ninth week infection decreased to zero (pots 16–25). Again when the wet period was decreased to one and two weeks, infection

Table II.

Relation of Variation in Time of Wet Period to Infection.

Date: 25. i. 24 to 16. iv. 24. Variety: Arran Chief.

Pot Nos.	Condition of Soil during 12 weeks experiment — Week 1	2	3	4	5	6	7	8	9	10	11	12	Number of Plants: Heavily infected	Slightly infected	Clean
1– 5	–	–	–	–	–	–	–	–	–	–	–	–	0	0	5
6–10	–	–	–	–	W	W	W	W	W	W	W	W	5	0	0
11–15	–	–	–	–	W	W	W	W	–	–	–	–	5	0	0
16–20	–	–	–	–	–	–	W	W	W	W	–	–	0	3	2
21–25	–	–	–	–	–	–	–	–	W	W	W	W	0	0	5
26–30	–	–	–	–	W	W	–	–	–	–	–	–	2	1	2
31–35	–	–	–	–	–	–	W	W	–	–	–	–	0	0	5
36–40	–	–	–	–	–	–	–	–	W	W	–	–	0	0	5
41–45	–	–	–	–	W	–	–	–	W	–	–	–	4	1	0
46–50	–	–	–	–	W	–	–	–	–	–	–	–	1	2	2
51–55	–	–	–	–	–	–	W	–	–	–	–	–	0	1	4
56–60	–	–	–	–	–	–	–	–	W	–	–	–	0	0	5
61–65	–	–	–	–	–	–	–	–	–	–	W	–	0	0	5
66–70	–	–	–	–	W	W	W	W	W	W	W	W	4	1	0

– =fairly dry soil. W=soil kept very wet.

Table III.

Relation of Variation in Time of Wet Period to Infection.

Date: 22. ii. 24 to 13. vi. 24. Variety: Arran Chief.

Pot Nos.	Condition of Soil during 16 weeks experiment — Week 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Number of Plants: Well infected	Slightly infected	Clean
1– 5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0	5
6–10	–	–	–	–	W	W	W	W	W	W	W	W	–	–	–	–	5	0	0
11–15	–	–	–	–	W	W	W	W	–	–	–	–	–	–	–	–	4	0	1
16–20	–	–	–	–	–	–	W	W	W	W	–	–	–	–	–	–	4	1	0
21–25	–	–	–	–	–	–	–	–	W	W	W	W	–	–	–	–	2	3	0
26–30	–	–	–	–	W	W	–	–	–	–	–	–	–	–	–	–	4	0	1
31–35	–	–	–	–	–	–	W	W	–	–	–	–	–	–	–	–	1	0	4
36–40	–	–	–	–	–	–	–	–	W	W	–	–	–	–	–	–	1	2	2
41–45	–	–	–	–	W	–	–	–	W	–	–	–	–	–	–	–	2	3	0
46–50	–	–	–	–	W	–	–	–	–	–	–	–	–	–	–	–	0	0	5
51–55	–	–	–	–	–	–	W	–	–	–	–	–	–	–	–	–	0	2	3
56–60	–	–	–	–	–	–	–	–	W	–	–	–	–	–	–	–	0	0	5
61–65	–	–	–	–	–	–	–	–	–	–	W	–	–	–	–	–	0	0	5
66–70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0	5
71–75	–	–	–	–	W	W	W	W	W	W	W	W	–	–	–	–	4	0	1

– =fairly dry soil. W=soil kept very wet.

was slight when this period came in the fifth to the seventh week, and was entirely absent when it came later. Two wet weeks with a dry interval between (pots 41–45) resulted in infection which though slightly less than in pots 6–10 was fairly heavy.

These results again demonstrate the importance of moisture in controlling infection by wart disease of potatoes. They show that when the soil is kept wet for one period only this must last for about a month if it is to produce a high percentage of infected plants. Two wet periods of one week produced almost as much infection. Again, it is shown that a high degree of moisture during the middle period of growth is more effective in promoting infection than in the later period, but the significance of this result is not yet evident.

After these plants had been removed the same soil was kept "fairly dry" in the same pots for one month and then fresh tubers were planted, care being taken to avoid reinfection of one pot from another. When most of the shoots had appeared above the surface of the soil all the pots were kept very wet for two months, after which the plants were examined. Every plant was found to be well infected with wart disease. The capacity for infection was thus shown to be latent in the soil in every pot and to be controlled simply by moisture conditions. The earlier varied treatment does not appear to have materially affected the capacity for later infection.

A duplicate of the experiment whose results are shown in Table II, was set up one month after it had begun (Table III), and while the second experiment was still in progress the results of the first were obtained. The causes operative in producing so much greater infection when the wet period occurred in the second month than when it occurred in the third are not clear. The possibility was considered that when the wet period was in the third month infection might have taken place, but that the diseased growths might not have had time to develop before the plants were examined. In order to test this possibility the second set of pots, which up to the twelfth week was treated similarly to the first, was left for a further interval of four weeks, during which the soil in all the pots was kept fairly dry. The plants were then taken up and examined. In this way an extra month was allowed in which infected tissue might develop.

The results shown in Table III are in general similar to those obtained in the earlier experiment, but show a general tendency to greater infection. When the wet period of four or two weeks occurred in the third month (pots 21–25 and 36–40) some plants were found to be infected,

indicating that lack of time for development partially accounted for the complete absence of infection in similarly treated plants in the previous experiment (Table II, pots 21–25 and 36–40). The fact, however, that infection in pots 21–25 and 36–40, Table III, was less than in the previous experiment in pots 11–15 and 26–30, Table II, in which the same time was allowed for growth subsequent to the wet period may require further explanation. It is possible that in the second month the potato plant may be in a more susceptible stage of growth than it is four weeks later; it may, for example, be actively forming young shoots or tubers which in the period of rapid growth are readily infected. Or again, there may be some kind of periodicity in the germination of the sporangia which enables them to germinate more readily at certain times than at others. This point requires further investigation.

Moisture estimations made on the soils in pots 1–5 and 6–10 showed that the "dry" soil has a moisture content in the region of 5 per cent., and the "very wet" soil about 33 per cent.

From a practical standpoint these results show that a high degree of infection by wart disease can be obtained on potatoes growing in pots when the soil is kept very wet for the second month and fairly dry for the first and third months. There is also a distinct indication which requires further study, that if the soil is kept wet and dry alternately a high degree of infection may be obtained. These two results are of importance for soil sterilisation research in which it may be undesirable to keep the soil in a wet condition throughout the experiment because this may interfere with the normal reactions of chemicals in the soil.

(b) *Type of Soil.*

A second factor likely to influence the degree of infection is the physical character of the soil and therefore the following observations are of interest. A number of pots containing Arran Chief tubers were set up, using different types of equally infected soil. No significant differences could be detected in the amount of infection present (Table IV) and a high percentage of the plants grown in each type of soil were diseased. The heavy Rothamsted soil has long been recognised as too heavy to give satisfactory results in small pots without the admixture of some lighter material. When however this soil was used in a large pot, 12×18 inches, the resulting infection was as heavy as in the lighter soils. It thus appears that heavy infection can be obtained in soils varying in physical character from sand to a heavy field soil.

Table IV.

Numbers of Plants Infected in Different Types of Soil.

Date: June–Sept. 1923.

Soil	Number of plants		Percentage of plants infected
	Clean	Infected	
Sand	4	36	90
Light sandy soil from Ormskirk	1	9	90
Medium loam from Rothamsted allotments	3	17	85
Soil mixture consisting of field soil 37·5 % „ „ sand 50 % „ „ cavings 12·5 %	4	66	94

(c) *Seasonal Factors.*

The possibility that some "seasonal" factor or factors may be operative in controlling infection was suggested by certain results obtained in the earlier part of this work, the indications being of the following kind. Arran Chief tubers were planted ten at a time at monthly intervals from November, 1921, to March, 1922, each tuber being in a separate pot containing infected soil. The plants were grown in the glasshouse and the soil was kept moist but not very wet. Similar pots containing tubers planted in the same way from April to July, 1922, were put out of doors, where the unusually heavy rainfall of that season kept the soil extremely wet. After three or more months' growth the plants were examined and it was found that those planted from November to March and maintained in the glasshouse were only slightly infected, the numbers of plants showing signs of wart disease varying from none to four with an average of two out of each set of ten. Those planted from April to July and grown out of doors were, however, heavily infected, the disease appearing in at least eight out of each set of ten plants. These results suggested either a seasonal effect or the presence out-of-doors of some condition favourable to infection which was not operative in the glasshouse. The results set out in Table I, obtained while this series was in progress, showed that the presence or absence of a high degree of soil moisture might well be the controlling factor and explain what, superficially, might be interpreted as a seasonal effect. While the former appeared to be the more probable cause of variation in infection, the possibility of a seasonal factor being operative could not be ignored.

A series of monthly plantings were therefore carried out in 1923 and again in 1924. The sporangial material for use in the 1923 set was collected in the field during the summer and autumn of 1922, dried and

sieved all together and left exposed to light and air until required. In the same way the sporangia for use in 1924 were collected in the late summer of 1923 and treated similarly. Each month a measured quantity of this sporangial material was mixed with 30 lb. of soil and divided between ten pots in each of which one Arran Chief tuber was planted. After the first two or three weeks the soil was kept very wet for the remaining ten or nine weeks when the plants were examined. The pots were all kept in the glasshouse during the whole period.

The results obtained in 1923 and 1924 are shown in Table V. Heavy infection was found in every plant set up from December, 1922, to May, 1923, after which there was a slight (and so far unexplained) falling off in infection which may have been due to ageing of the sporangia. This hypothesis is supported by the fact that a higher percentage, 80–100 per cent., infection is obtained in the summer when new sporangia are used. The tubers planted after August, which were of the same stock as those planted earlier, were much shrivelled and made such poor growth that no reliable infection results could be obtained from them. No results were derived therefore from plantings in September, October or November. The results obtained in 1924 were similar to those of 1923.

Table V.

Variety: Arran Chief.

Date		Number of plants	
Month planted	Month examined	Clean	Infected with wart disease
Sporangia collected autumn 1922.			
21. xii. 22	15. iii. 23	0	10
18. i. 23	12. iv. 23	0	9
15. ii. 23	10. v. 23	0	10
15. iii. 23	7. vi. 23	0	10
12. iv. 23	5. vii. 23	0	10
10. v. 23	22. vii. 23	0	10
7. vi. 23	30. viii. 23	2	8
5. vii. 23	27. ix. 23	4	6
9. viii. 23	25. x. 23	1	9
29. viii. 23	22. xi. 23	4	6
Sporangia collected autumn 1923.			
17. i. 24	10. iv. 24	0	10
14. ii. 24	1. vii. 24	2	8
13. iii. 24	3. vii. 24	0	10
10. iv. 24	2. vii. 24	0	10
5. vi. 24	1. ix. 24	2	8
3. vii. 24	26. ix. 24	1	9

It thus appears that the apparent seasonal effect obtained in 1922 was simply dependent on variation in moisture. No evidence of any seasonal factor has been obtained and it therefore appears probable that a high degree of infection can be obtained in pot experiments of this type carried out at any season of the year.

The Winter Sporangium.

The fate of the winter sporangium in the soil is a problem of fundamental interest. It has been shown in the field that soil infected with wart disease retains, in the absence of the potato plant, its capacity for causing infection for at least nine years (5). The mode of survival of the parasite in the absence of the host is however obscure. It is known that *Solanum nigrum* and *S. dulcamara* are susceptible to attack by the fungus. It is possible that other weeds may act as hosts, thus enabling the organism to reinfect soil periodically with fresh sporangia. On the other hand, the sporangia may remain dormant in the soil until the presence of a host acts as a stimulus to germination, in which case the known longevity of such reproductive bodies in the fungi renders it not surprising that the soil should remain infected for many years. A further hypothesis is that the sporangia germinate irrespective of the presence or absence of a susceptible host. The germination may be spread over a number of years or the zoospores from the germinating sporangium may resort to a saprophytic mode of life resuming their parasitic propensities when suitable host plants are present. Curtis (2), however, observed that the zoospores after their discharge from the sporangium cannot survive long *in vitro*, and found no evidence of saprophytic tendencies. The whole question is of considerable importance and we have very little evidence bearing upon it.

(a) *Age of Sporangia and Infection.*

The survival in the soil of sporangia under the conditions of pot experiment for over a year is shown by the following observation. Sporangial material collected in the autumn of 1922 was kept dry until it was mixed with soil in the usual manner in March, 1923. The soil was put into pots in some of which tubers were planted immediately. After three months these were examined and found to be infected with wart disease. No tubers were put into the remaining pots but the soil was kept watered regularly and weeded periodically for a year. The soil was then allowed to become dry and remained so for some months. Tubers planted in these pots at the end of July, 1924, were examined

in October and found to be particularly heavily infected. The results are set out in Table VI. No diminution in the infecting power of the soil could be observed.

Table VI.

Survival of Winter Sporangia in the Soil.

Date		Number of plants	
Soil infected	Tubers planted	Clean	Infected with wart disease
16. iii. 23	16. iii. 23	0	10
16. iii. 23	31. vii. 24	0	9

Further evidence of the prolonged retention of vitality by the sporangium in the soil is shown by another observation made on sporangia collected in the summer of 1923. The sporangial material was kept dry until it was used to infect soil in October, 1923. The tubers were old and grew so poorly that only very slight infection was present. The soil was kept in the pots and replanted with tubers in July, 1924. On examination in October they were all found to be extremely heavily infected. The sporangia had thus remained in the soil in pots for about ten months with no apparent loss in vitality. It is however doubtful whether they retain their vitality so long when kept dry out of the soil. This point needs further investigation.

(b) *Dormancy in Winter Sporangia.*

A further point of some interest lies in the time necessary to obtain infection with winter sporangia. It has been observed in all the pot experiments hitherto carried out that no signs of infection appear until about two months after the soil has been infected and the tubers planted. Minute warts about $\frac{1}{8}$ inch in diameter are found at the end of this period and these by the end of another month have developed into large warted masses often one or more inches in diameter. The rapidity of growth suggests that the warts observed at the end of the second month are newly formed. It would appear therefore that either the sporangia lie dormant in the soil for a period before germinating and infecting the host, or that the potato plant is less liable to infection in the earlier stages of its growth.

The following test was arranged to throw some light on this question. Uniformly infected soil was put into 20 pots. Tubers were planted in only ten of these (pots 1–10), but all the 20 pots were equally well watered and treated similarly for one month. At the end of this time

tubers were planted in pots 11–20 and another set of ten pots (21–30) were filled with soil freshly infected and immediately planted with tubers. After a further period of one month all the potatoes in the whole of the 30 pots were examined. The results are shown in Table VII. The plants in pots 1–10, which had then grown for two months in the soil infected for the same period, showed small warts on nine out of ten plants. No infection appeared in the plants grown for one month in soil infected for one month (pots 21–30). The potato plants which had grown for one month in soil which had been infected for two months all had small warts on them (pots 11–20). These warted areas were about the same size as those obtained by infection from summer sporangia in about 14 days, so that infection may be assumed to have taken place within this period.

Table VII.

Dormancy of Sporangia.

Variety: Arran Chief.

Pot Nos.	Date: Soil infected	Date: Tuber planted	Date: Plants examined	Length of time since: Soil infected	Length of time since: Tuber infected	Number of plants: Clean	Number of plants: Infected with wart disease
1–10	16. iii. 23	16. iii. 23	11. v. 23	2 months	2 months	1	9
11–20	16. iii. 23	13. iv. 23	11. v. 23	2 „	1 month	0	10
20–30	13. iv. 23	13. iv. 23	11. v. 23	1 month	1 „	10	0

It thus seems clear that under these conditions the lapse of time before warts become evident depends, not on the stage of growth of the potato, but on the germination of the sporangium. There appears to be a definite time lapse in the region of six weeks after the sporangia are put into the soil before germination can take place. A similar but slightly longer delay in germination (ten weeks) was noted by Miss Curtis(2), dealing with sporangia *in vitro*. This lag in germination may depend on the time required for maturation of the sporangium, on the rate of penetration of the cell wall by water or on other less obvious factors, but the problem yet remains to be investigated.

(c) *Sporangial Numbers.*

Some knowledge of the relationship between the actual numbers of sporangia in the soil and the incidence of infection, is of considerable practical importance in pot experiments. In the following test carried out to throw some light upon this relationship, the sporangial material consisted of decayed warts and wart-bearing tubers, air dried, ground

down and passed through a sieve of 3 mm. mesh. Various quantities of the resulting powder were measured by volume and thoroughly mixed with 30 lb. of soil, which was then divided between ten pots in each of which one tuber was placed. An estimation of the actual numbers of sporangia per cubic centimetre of sporangial material was made as follows: The average weight of 1 c.c. of sporangial powder was found to be 0·825 gm. This quantity was prepared in a finely divided condition and the actual numbers of sporangia present estimated by the dilution method. This showed the numbers of sporangia present in 1 c.c. of dry sporangial powder to be in the region of 105,000. Table VIII shows the sporangial numbers added to 30 lb. of soil, and the numbers calculated per gram of soil.

Table VIII.

Relation of Numbers of Sporangia in the Soil to Infection.

Date: 12. iii. 23 to 4. vi. 23. Variety: Up-to-Date.

Volume of sporangial material. Mixed with 30 lb. soil	Numbers of sporangia	Numbers of sporangia per gram of soil	Number of plants	
			Clean	Infected with wart disease
200 c.c.	21,000,000	46,200	0	10
100 ,,	10,500,000	23,100	0	10
50 ,,	5,250,000	11,550	0	10
25 ,,	2,625,000	5,775	0	10
10 ,,	1,050,000	2,310	1	9
5 ,,	525,000	1,155	3	7
1 ,,	105,000	231	9	1

Variation in the quantity of sporangial material from 200 c.c. down to 25 c.c. produced no apparent diminution in the amount of infection, while from 25 c.c. down to 1 c.c. there was a rapid decline in the numbers of plants showing disease.

Relative Susceptibility of Different Varieties of Potato.

Great variation in the degree of susceptibility to wart disease has been observed in the field among different varieties of potato. It is of interest to learn, whether this difference in susceptibility is still evident under the more uniform conditions of pot experiment and, further, whether varieties which are only slightly susceptible in the field will show infection under these conditions. Ten different varieties of potato susceptible to wart disease were therefore grown in pots of heavily infected soil in the usual way. The varieties have been grouped by Mr H. Bryan, Superintendent of the Potato Testing Station, Ormskirk,

into those which in the field appear to be highly susceptible, moderately susceptible and slightly susceptible. A high percentage infection was obtained even in the least susceptible varieties grown in pots. The results are shown in Table IX and demonstrate that under conditions very favourable to infection a high percentage of even the least susceptible variety becomes warted.

Table IX.

Infection of Ten Different Susceptible Varieties of Potato grown in heavily infected Soil in Pots.

Date set up 17. i. 24.

Variety	Susceptibility in the field	Number of plants		Percentage plants infected	Average
		Clean	Infected		
Arran Chief	Great	0	19	95	90 %
President	„	1	4	80	
Up-to-Date	„	1	4	80	
Epicure	Moderate	0	5	100	87 %
May Queen	„	0	5	100	
Ninety-fold	„	2	3	60	
Sir John Llewelyn	„	1	4	80	
Sharpe's Express	„	1	4	80	
Puritan	„	0	5	100	
British Queen	Slight	2	8	80	80 %

When however the soil was only slightly infected and the conditions were therefore less favourable to infection by wart disease, variation in degree of susceptibility became evident (Table X). The amount of infection was, in general, reduced and the incidence of disease was smallest in those varieties which proved least susceptible in the field. Thus under conditions which are not very favourable to infection, variation in the relative susceptibility of different varieties is evident and is found to be broadly similar to that observed in the field.

The possibility that under conditions which bring about a high percentage of infection in susceptible varieties immunity itself might break down, could not be overlooked. A further test was therefore carried out in which tubers of 12 immune and 12 susceptible varieties were grown in heavily infected soil under identical conditions. The results given in Table XI show that a high percentage of tubers belonging to susceptible varieties were infected, while all the immunes which on the whole showed better growth, and were therefore more liable to show disease, were free from infection.

From a practical point of view it is useful to know that in well-

Table X.

Infection of Ten Different Susceptible Varieties of Potato grown in slightly infected Soil.

Date: 12. vi. 23 to 19. ix. 23.

Variety	Susceptibility in the field	Number of plants: Clean	Number of plants: Infected	Percentage plants infected in each class
Arran Chief	Great	5	5	60 %
Up-to-Date	,,	4	6	
King Edward	,,	1	4	
Epicure	Moderate	4	1	21 %
Duke of York	,,	5	0	
Ninety-fold	,,	3	1	
Sir John Llewelyn	,,	4	1	
Sharpe's Express	,,	3	2	
		4	1	
British Queen	Slight	5	0	10 %
Harbinger	,,	4	1	

Table XI.

Infection Experiments with Susceptible and Immune Varieties of Potato.

Date: 27. vi. 24 to 5. ix. 24.

Variety	Susceptibility under field conditions	Number of plants: Clean	Number of plants: Infected with wart disease	Average
Arran Chief	Great	0	10	93 %
King Edward	,,	2	7	
President	,,	0	5	
Up-to-Date	,,	0	5	
Epicure	Moderate	0	10	97 %
Queen Mary	,,	0	5	
Ninety-fold	,,	0	5	
Sir John Llewelyn	,,	0	5	
Sharpe's Express	,,	0	5	
Puritan	,,	1	7	
Harbinger	Slight	0	10	93 %
Mein's Early Round	,,	1	4	
Arran Comrade	Immune	10	0	0%
Roderick Dhu	,,	10	0	
Arran Comrade	,,	10	0	
Katie Glover	,,	10	0	
Tinwald Perfection	,,	10	0	
Golden Wonder	,,	10	0	
Di-Vernon	,,	10	0	
The Ally	,,	10	0	
Immune Ashleaf	,,	5	0	
Crusader	,,	5	0	
America	,,	5	0	
Majestic	,,	5	0	
The Bishop	,,	5	0	

infected soil, other conditions being favourable, infection of a high proportion of even slightly susceptible potatoes can be obtained in pot experiments, and that by this method immune varieties can be distinguished from susceptible ones. This test appears to be at least as reliable as field trials; it can be carried out more rapidly than such trials and does not depend for success on suitable seasonal conditions.

Alternative Hosts of *Synchytrium endobioticum*.

The possibility has been suggested that plants other than the potato act as hosts for *Synchytrium endobioticum* and thus help both to disseminate the disease and to prolong its existence in the soil in the absence of the potato plant. Cotton(1) has shown that *Solanum nigrum* and *S. dulcamara* are slightly susceptible to attacks by the fungus and Kunkel and Orton(4) that this is also true of certain varieties of tomato.

The infection of hosts other than the potato has here been attempted by the method of pot experiment described. The tomato plants were grown from seed sown in infected soil and then planted out in separate pots. The soil was heavily infected with *Synchytrium* sporangia and to facilitate infection, especially of the lower axillary shoots, the plants were set deeply so that several inches of the main stem were covered with soil. Macerated warts were mixed with water and poured round the stem while planting. Table XII shows the results of such tests obtained in 1922 with tomatoes and other plants of the family Solanaceae. Small warts measuring about 0·5 × 0·3 cm. in which winter sporangia were present were found on three plants of the variety Manx Marvel and one wart measuring 1·0 × 1·0 cm. on one plant of the variety Blaby. No trace of wart disease could be found on any of the other five varieties of tomato. Warts were also found on two out of four plants of *Solanum nigrum*, the largest measuring about 1·0 × 0·5 cm.

A similar test was carried out in 1923, the results of which are also shown in Table XII. Small warts, the largest measuring about 0·3 cm. diameter, were found on two varieties of tomato, Blaby and Fillbasket, and on four plants of *S. dulcamara*. The susceptibility of *S. nigrum* and *S. dulcamara* is thus confirmed and that of certain English varieties of tomato demonstrated. In all cases, however, the degree of susceptibility appears to be very slight.

In 1924, at the request of the Ministry of Agriculture and Fisheries, tests were carried out to ascertain whether certain plants belonging to the Solanaceae, which had been suspected of carrying wart disease, are susceptible to invasion by *Synchytrium endobioticum*. Extensive tests were made with *Salpiglossis sinuata*, *Atropa belladonna*, *Hyoscyamus*

niger and *Lycium chinense*, while control pots containing tubers of both the very susceptible variety Arran Chief and the slightly susceptible Harbinger were set up. The ten control Arran Chief plants were well infected and nine out of ten Harbingers. All the plants belonging to the four species tested were thoroughly examined but appeared to be entirely free from wart disease.

Finally it may be stated that during the carrying out of these pot experiments a considerable number of weeds have been examined for traces of infection by wart disease. Also during extensive field experiments extending over four years a close watch has been kept by Dr Brierley and myself on the weed flora of infected soil. Growths or appearances that suggested infection by wart disease were critically examined but in no case was wart disease found to be present.

It thus appears that slight infection by wart disease has been

Table XII.

Infection Experiments with Plants of the Family Solanaceae.

Name of plant	Date	Number of plants: Clean	Number of plants: Infected with wart disease
Tomato. Ailsa Craig	Planted May. Examined Oct. 1922	10	0
„ Blaby		9	1
„ Blako		8	0
„ Buckley		5	0
„ Comet		7	0
„ Fillbasket		5	0
„ Manx Marvel		5	3
Solanum nigrum		2	2
Datura Stramonium		5	0
Hyoscyamus niger		1	0
Tomato. Ailsa Craig	Planted June. Examined Oct. 1923	1	0
„ Blaby		8	1
„ Blako (Blaby × Kondine Red)		6	0
„ Buckley		6	0
„ Fillbasket		4	1
„ Improved Comet		5	0
„ Kondine Red		5	0
„ Manx Marvel		1	0
Solanum nigrum		5	0
„ *dulcamara*		1	4
Salpiglossis sinuata	Planted June. Examined Sept. 1924	25	0
Hyoscyamus niger		25	0
Atropa belladonna		25	0
Lycium chinense		25	0

observed in *Solanum nigrum* and *S. dulcamara*, and on certain varieties of tomato, all plants belonging to the family Solanaceae. Extensive investigation of other possible hosts apart from the potato has revealed no trace of wart disease.

INFECTION BY SUMMER SPORANGIA.

A consideration of the life-history of *Synchytrium endobioticum* suggests that infection by means of the summer sporangia, which germinate immediately, would be far more rapid than by the winter sporangia, which have a dormant period of many weeks before germination. When this occurs the winter sporangia liberate zoospores which infect growing potato tissue, resulting in a rapid development of sori of summer sporangia. These mature and in the presence of water are discharged, very soon liberating swarm-spores which either singly or after fusion in pairs infect fresh potato tissue, producing in the first case more summer sporangia, thus rapidly spreading the area of infection in adjacent potato tissue, or in the second case winter sporangia, which carry on the disease to the following season.

The inoculation of a clean growing shoot by means of newly discharged summer sporangia or by the zoospores set free from them seemed likely to provide a rapid method for producing infection.

Technique.

After some unsuccessful attempts to bring about infection by this means an easy and successful technique was devised which was carried out as follows: Fresh growing warts bearing summer sporangia were pinned on to tubers, in close contact with the young growing shoots to be infected. It was found to be of vital importance in obtaining infection that a film or drop of water should connect the warted tissue and the shoot to be infected practically all the time. The following simple apparatus was found to be effective. The tubers with attached warts are placed on a large piece of filter paper dipping into a vessel of water and supported over it by means of a wire frame. The tubers are well sprayed with water from a fine sprayer once or twice a day, are then covered with a sheet of damp filter paper and the whole covered with a bell-jar. In this way about a dozen tubers can be tested together. The infecting warts are renewed every seven days. By this means infection was obtained in most tubers, as described in the following experiments, in about 21 days. If the test is to be carried out on an extensive scale daily spraying can be avoided by the maintenance of an almost saturated

atmosphere around the tubers to be infected, *e.g.* by placing them after spraying under a bell-jar supported above a water bath kept at about 30° C. The tubers must be several (about 9″) inches above the water, otherwise the moisture tends to evaporate from them. In this way the wet film connecting wart and shoot need only be renewed by spraying about once in every few days.

EXPERIMENTAL.

Typical results obtained with susceptible varieties are shown in Table XIII. Summer sporangia were observed in many cases after 14 days. Occasionally in this work they have been detected after so short a period as seven days. After 21 days most tubers bore warts and after 28 days winter sporangia had developed, and could be detected on every susceptible tuber. No difference in degree of relative susceptibility of different varieties were observed.

Table XIII.

Infection of Susceptible Varieties by Summer Sporangia.

Date: 19. iii. 24 to 16. iv. 24.

Variety	Susceptibility to wart disease in the field	Total no. of tubers	No. tubers infected after 7 days	14 days	21 days	28 days
Arran Chief	Great	10	0	2	10	10
King Edward	,,	5	0	0	4	5
Up-to-Date	,,	5	0	4	5	5
Epicure	Moderate	3*	0	2	3	3
Sharpe's Express	,,	5	0	2	5	5
Ninety-fold	,,	5	0	3	5	5
Puritan	,,	10	0	3	10	10
Mein's Early Round	Slight	8*	0	1	8	8
Harbinger	,,	10	0	3	10	10
British Queen	,,	10	0	5	10	10

* Two out of the original five and ten tubers decayed.

If this method is to be of use in distinguishing immune from susceptible tubers, it must be shown that immunity does not break down under these conditions. The following trials were set up to test this question and Table XIV shows the results of infection experiments using both immune and susceptible varieties. After four weeks every susceptible tuber was infected with wart disease, while no signs of infection could be found on any of the immune varieties. Some of these were kept under treatment for another week but still showed no trace of infection.

Table XIV.

Inoculation of Susceptible and Immune Varieties by Summer Sporangia.

Date: 23. vi. 24 to 21. vii. 24.

Variety	Susceptibility to wart disease in the field	Total no. of tubers	No. of tubers infected after		
			14 days	21 days	28 days
Arran Chief	Great	4	0	4	4
King Edward	„	5	0	5	5
President	„	5	2	4	5
Up-to-Date	„	5	0	1	5
Epicure	Moderate	5	0	4	5
Sir John Llewelyn	„	4	1	1	4
Harbinger	Slight	3	2	2	3
Arran Comrade	Immune	5	0	0	0
Roderick Dhu	„	5	0	0	0
Katie Glover	„	5	0	0	0
Tinwald Perfection	„	5	0	0	0
Golden Wonder	„	5	0	0	0
Di-Vernon	„	5	0	0	0
The Ally	„	5	0	0	0
Immune Ashleaf	„	4	0	0	0
Crusader	„	5	0	0	0

CONCLUSIONS.

The need for a method of pot experimentation to serve as a basis for soil sterilisation research, which is now carried on almost wholly in the field and for a method of testing the immunity or susceptibility of new varieties of potato, has been indicated. The length of time involved in the field trials for immunity and susceptibility, which is at least two years, causes great delay in arriving at the results of genetic research and in putting new commercial varieties on the market.

The study of conditions controlling infection of potatoes by the winter sporangium of *Synchytrium endobioticum* in the soil in pots has shown that a high percentage of even the least susceptible varieties become infected when the right conditions are maintained. It is thus possible to carry out experiments with potatoes in small pots containing only about three pounds of heavily infected soil, and to obtain 80–100 per cent. of the plants infected. Soil moisture is a very important factor in controlling infection which is general and heavy only when the soil is very wet for a considerable part of the growth period. Pot experiments of this type can be completed in three months and can probably be carried out at almost any time of the year. They should therefore be useful in soil sterilisation and other research, and may be of use in testing for immunity.

A method of infection by summer sporangia has been devised which is capable of modification for use on an extensive scale for testing the immunity or susceptibility of new varieties. It can be carried out in a period of three or four weeks and apparently at any time of the year.

SUMMARY.

A study of certain conditions controlling infection of potatoes by the winter sporangium of *Synchytrium endobioticum* in the soil and by the summer sporangium in the laboratory has been made with a view to finding a reliable method of pot experiment to serve as a basis in soil sterilisation research, and a method for testing immunity or susceptibility more rapidly than is at present done in the field.

Experiments on infection by the winter sporangium in the soil have shown that a very high degree of soil moisture is necessary to ensure infection but this need not be present during the whole of the growth period. It appears most effective when the wet period is in the second month.

A high percentage infection is obtained in potato plants grown in soils of very varying physical character.

Under the conditions of pot experiment the wart disease organism survives in the soil in the absence of the potato plant for a period of at least a year. There appears to be a dormancy period of about six weeks between soil infection and sporangial germination. The relation of numbers of sporangia in the soil to the incidence of disease is discussed.

When favourable conditions were maintained 80–100 per cent. of the plants tested were found to be infected within a period of three months, even in varieties which in the field appear least susceptible. Under conditions less favourable to infection the relative susceptibilities of the several varieties become clearly marked. No wart disease was found under any conditions on immune varieties.

Infection of various plants other than the potato was attempted. Small warts were found on three varieties of tomato and on *Solanum nigrum* and *S. dulcamara*, but none on five other varieties of tomato, on *Datura Stramonium*, *Salpiglossis sinuata*, *Hyoscyamus niger*, *Atropa belladonna*, *Lycium chinense* or on many common weeds grown in infected soil.

A method is described for infecting sprouting tubers with wart disease by means of summer sporangia. Susceptible varieties subjected to this treatment develop young warts within three weeks, while immunes remain clean. The method can therefore be used for testing immunity or susceptibility in the laboratory.

I am indebted to Dr W. B. Brierley for much helpful criticism received during the course of this work.

REFERENCES.

(1) Cotton, A. D. (1916). Host Plants of *Synchytrium endobioticum*. *Kew Bulletin*, 272.

(2) Curtis, K. M. (1920). The Life-History and Cytology of *Synchytrium endobioticum* (Schilb.), Perc., the Cause of Wart Disease in Potato. *Phil. Trans. Roy. Soc.* Ser. B, ccx, 409–478.

(3) Gimingham, C. T. and Spinks, G. T. (1919). Soil Sterilisation. *Univ. Bristol Agr. and Hort. Res. Sta. Ann Rep.* 37.

(4) Kunkel, L. O. and Orton, C. R. (1920). A new host for the Potato Wart Disease. *U.S. Dept. Agr. Dept. Circ. III*, 17–19.

(5) Schaffnit, E. and Voss, G. (1918). Versuche zur Bekämpfung des Kartoffelkrebses im Jahre 1917. *Zeitschr. f. Pflanzenkr.* xxviii, 111.

(6) (1922) Potato Wart. *Penn. Sta. Bul.* 170, 16.

(*Received September 29th*, 1924.)

Addendum.

A paper by Spieckermann and Kotthof received while the present communication was in press, published in Germany in March, describes another method for testing immunity and susceptibility. A compost rich in germinating winter sporangia, prepared from warted material kept out of doors for several weeks in the autumn and preferably exposed to frost, was laid on the eyes of sprouting pieces of tuber and kept very wet at a temperature of 16–20° C. for about three weeks. Infection was observed in all susceptible varieties, and after six weeks a few varieties previously regarded as immune showed slight traces of infection.

Spieckermann, A. and Kotthof, P. *Die Prüfung von Kartoffeln auf Krebstigkeit. Deutsche landw. Presse*, 1924, li. 11, p. 114–115.